Aspirin and the Prevention of Colorectal Cancer

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Introduction

Despite the efforts of population-based screening programs, colorectal cancer continues to rank as one of the leading causes of cancer-related mortality in economically developed countries and is responsible for over 690,000 deaths each year globally [1]. The majority of colorectal cancers arise from adenomatous polyps via the so-called traditional adenoma-carcinoma sequence [2], although the contribution of the serrated neoplasia pathway has been increasingly appreciated over the past decade [3]. Although there is robust evidence to support the efficacy of lower endoscopy and polypectomy in the reducing colorectal cancer incidence and mortality [4–6], the success of endoscopic screening has been limited by poor uptake, patient inconvenience, high cost, and some risk of morbidity [7, 8]. Furthermore, no currently available screening modality offers absolute protection against colorectal tumor development or colorectal cancer-related death. The concept of colorectal cancer chemoprevention, the use of natural or synthetic agents to prevent, suppress, or reverse carcinogenic progression to invasive cancer, has therefore gained popularity as an attractive preventive strategy [9, 10].

There is compelling evidence that aspirin (acetylsalicylic acid, ASA) and other nonsteroidal anti-inflammatory drugs (NSAIDs) prevent the development and progression of colorectal neoplasia [9, 10]. Aspirin has been marketed as an analgesic and antipyretic for over a century, and the pharmacologic use of salicylates spans millennia, with the use of willow leaves to alleviate fever described in the Ebers Papyrus of 1550 BCE [11]. With proven effectiveness in the prevention of cardiovascular events and worldwide aspirin production and consumption running at some 40,000 metric tonnes annually [12], aspirin has garnered an unparalleled clinical pedigree. Clinicians are eminently familiar with aspirin's toxicity and side effect profile, an asset that stands aspirin in good stead as chemoprevention candidate, particularly in light of adverse events encountered during clinical experience with more novel agents, such as the selective COX-2 inhibitors [13]. In this chapter we present a summary of the evidence that underpins the

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case for aspirin in the prevention of colorectal neoplasia, highlight putative mechanisms of action, and explore the factors that have thus far limited the widespread adoption of aspirin as a chemopreventive agent.

Aspirin and Sporadic Colorectal Cancer: Evidence from Observational Studies

Although initial reports on the effect of the NSAID, sulindac, on polyp burden in patients with familial adenomatous polyposis (FAP) had begun to emerge during the early 1980s [14], it was not until 1988 that evidence for an association between aspirin and sporadic colorectal cancer was demonstrated [15]. In a case-control analysis of data from the Melbourne Colorectal Cancer Study, which examined associations between medications, chronic illnesses and operations, and colorectal cancer risk, a lower frequency of colorectal cancer was observed in those who used aspirin or aspirin-containing medications (odds ratio [OR], 0.53; 95 % confidence interval [CI], 0.40–0.71). This finding was unexpected, and the authors urged early replication given the potential implications for cancer chemoprevention [15]. However, results of the next major study of aspirin and cancer risk, published in 1989, were conflicting. In a prospective cohort of 13, 987 elderly residents of a retirement community in California, daily aspirin use was associated with a modestly increased risk of incident colon cancer (relative risk [RR], 1.5; 95 % CI, 1.1–2.2) over six and a half years of follow-up [16]. Large-scale, population-based, prospective data for aspirin and colon cancer mortality were first published in 1991, derived from the US Cancer Prevention Study II (CPSII) [17]. In this analysis of 662,424 men and women, the use of aspirin 16 or more times per month for at least 1 year was associated with a 40 % reduction in colon cancer mortality over 6 years of follow-up (hazard ratio [HR], 0.60; 95 % CI, 0.40–0.89) [17]. In a subsequent cancer incidence analysis, conducted within a subset of CPSII participants [18], daily use of standard-dose aspirin ($\geq$325 mg) for at least 5 years was associated with a RR for colorectal cancer of 0.68 (95 % CI, 0.52–0.90). Similar associations have been observed in other large population-based cohort studies. In an analysis of 47,363 male US health professionals in the Health Professionals Follow-Up Study (HPFS), who were followed up over 18 years, regular use of aspirin (at least twice per week) was associated with a 21 % reduction in colorectal cancer risk (RR, 0.79; 95 % CI, 0.69–0.90) [19]. A similar magnitude of risk reduction was obtained for women in an analysis of 82,911 participants of the Nurses' Health Study (NHS) [20]. During 20 years of follow-up, the use of two or more standard-dose aspirin tablets per week was associated with a 23 % reduction in colorectal cancer risk (RR, 0.77; 95 % CI, 0.67–0.88) [20]. In a separate analysis of 79,439 women enrolled in the NHS, current aspirin use was associated with a 28 % reduction in colorectal cancer mortality and a 25 % reduction in all-cause mortality [21]. In an additional large US cohort of older men and women, the NIH-AARP Diet and Health Study ($N = 301{,}240$), compared to no aspirin use, a reduction in incident colorectal cancer was observed in association with daily or weekly use of aspirin over the preceding 12 months (HR, 0.88 and 95 % CI, 0.80–0.97, and HR 0.86 and 95 % CI, 0.79–0.94, respectively) [22].

An inverse association between aspirin use and colorectal cancer risk has been observed in several smaller cohort studies and in a number of case-control analyses [23]. A meta-analysis of 26 case-control studies generated a pooled risk estimate of 0.67 (95 % CI, 0.60–0.74) for any aspirin use and 0.62 (95 % CI, 0.58–0.67) for the maximum category of aspirin intake across 17 studies that stratified by aspirin intake [23].

Finally, in addition to an association between aspirin use and risk of incident or fatal colorectal cancer, observational data also suggest that pre-diagnostic aspirin use may be associated with disease stage at presentation. In a meta-analysis of five cohort studies that included information on stage, regular aspirin use was associated with a reduced risk of cancers with distant metastases (OR, 0.69; 95 % CI, 0.57–0.83), but not with the likelihood of regional spread [23].

Data from Randomized Trials of Aspirin in the Prevention of Cardiovascular Events

Linking aspirin exposure during cardiovascular trials, where treatment was assigned rather than self-selected, to long-term outcomes represents a valuable research approach. Details of the major cardiovascular trials of aspirin are summarized in Table 14.1. Rothwell and colleagues evaluated cancer incidence and mortality in randomized trials of aspirin from the UK and Sweden, where post-trial outcome data could be reliably obtained from death and cancer registries [24]. Two primary prevention studies fulfilled the inclusion criteria of minimum recruitment of 1000 participants and treatment duration of least 2.5 years: the British Doctors Aspirin Trial (BDAT) [25], which recruited apparently healthy male physicians, and the Thrombosis Prevention Trial (TPT) [26], which identified men with high cardiovascular risk scores through their primary care physicians. Two secondary prevention trials were included: the Swedish Aspirin Low-Dose Trial (SALT) [27] and the UK-TIA Aspirin Trial [28], which examined women and men with a history of cerebrovascular disease or retinal artery occlusion. Aspirin dose in the treatment arms of these trials varied from 75 mg to 1200 mg daily, and the median duration of scheduled treatment ranged from 2.6 to 6.9 years [24]. Among a total of 14,033 participants randomized to aspirin or control, there were 397 documented colon and rectal cancers in 391 individuals, including 240 fatal cases. In a pooled analysis of individual patient data from the four trials, allocation to aspirin reduced colorectal cancer incidence by 24 % and colorectal cancer-specific mortality by 35 %, over a median of 18.3 years of follow-up [24]. Rothwell and colleagues subsequently conducted a further pooled analysis, incorporating individual patient data from eight trials where the mean scheduled aspirin treatment was at least 4 years (BDAT, UK-TIA, TPT, Early Treatment Diabetic Retinopathy Study [ETDRS] [29], Swedish Angina Pectoris Aspirin Trial [SAPAT] [30], Japanese Primary Prevention of Atherosclerosis with Aspirin for Diabetes [JPAD] study [31], Prevention of Progression of Arterial Disease and Diabetes [POPADAD] study [32], and Aspirin for Asymptomatic Atherosclerosis [AAA] trial [33]). Among 25,570 participants, there were 674 within-trial cancer-related deaths. Assignment to aspirin at doses ranging from 75 mg to 1200 mg per day was associated with a statistically significant 21 % reduction in cancer-related mortality [34]. In an analysis restricted to data from the six trials that included site-specific cancer data, the HR for risk of death from colorectal cancer ($N = 54$) among those assigned to aspirin was 0.41 (95 %, CI, 0.17–1.00) after at least five years of follow-up. For BDAT, UK-TIA, and TPT, up to 20 years of extended posttrial follow-up was obtained; for an aspirin treatment duration of 5 years or longer, the pooled colorectal cancer mortality HR over 20 years was 0.69 (95 % CI, 0.45–0.81) [34].

The effect of aspirin on the risk of cancer metastases has also been examined by exploiting data from five UK cardiovascular randomized trials of daily aspirin [35]. Among 17,285 participants, there were 775 in-trial incident solid cancers for which the metastasis status was known. Compared to the control groups, those randomized to aspirin had an OR of 0.59 (95 % CI, 0.44–0.78) for metastases from all solid tumors and an OR of 0.36 (95 % CI, 0.18–0.74) for colorectal cancer metastases [35]. These data are consistent with observational data for regular aspirin use [23] and suggest that an effect of aspirin on cancer metastasis may partly explain the greater reduction in CRC fatality relative to CRC incidence observed in the meta-analysis of long-term effects of aspirin in randomized trials [24].

While these data on aspirin and cancer are certainly persuasive, it should be remembered that these were secondary analyses of cardiovascular prevention trials. Thus, the capture of within-trial cancer and cancer-related deaths may be less reliable compared to studies where cancer outcomes were primary endpoints. Furthermore, where post-trial follow-up was possible, ascertainment of outcomes was dependent on linkage with registry entries, and data on exposure to aspirin, NSAIDs, or cancer

Table 14.1 Cancer outcomes in major cardiovascular trials of aspirin[a]

Trial	Participants (active/control)	Placebo controlled, double blind	Population	Aspirin dose	Median treatment duration (years)	Result (95 % CI)		
						CRC incidence	CRC mortality	Mortality from any cancer
BDAT	3429/1710	No	Primary prevention of CVD in male physicians	500 mg daily vs. control	6.0	HR 0·70 (0·51–0·97)	OR 0·73 (0·49–1·10)	OR 0.79 (0.55–1.14)
TPT	2545/2540	Yes	Primary prevention of CVD in men at increased risk of vascular events	75 mg daily vs. placebo	6.9	HR 0.75 (0.56–0.97)[b]	OR 0·61 (0·40–0·94)	OR 0.83 (0.62–1.11)
SALT	676/684	Yes	Secondary prevention of CVD following TIA or minor ischemic stroke	75 mg daily vs. placebo	2.7		OR 0·71 (0·27–1·86)	OR 0.71 (0.27–1.86)
UK-TIA	811/821/817	Yes	Secondary prevention of CVD following TIA or minor ischemic stroke	300 mg vs. 1200 mg daily vs. placebo	4.4	HR 0·82 (0·49–1·38)	OR 0·50 (0·21–1·17)	OR 0.45 (0.25–0.82)
ETDRS	1856/1855	Yes	Primary prevention of CVD and renal disease in patients with diabetes	650 mg daily vs. placebo	5.0	–	–	OR 1.14 (0.56–2.35)
SAPAT	1009/1026	Yes	Primary prevention of myocardial infarction in patients with stable angina	75 mg daily vs. placebo	4.2	–	–	OR 0.53 (0.25–1.15)
JPAD	1262/1277	Yes	Primary prevention of CVD in patients with type 2 diabetes	81 or 100 mg daily vs. placebo	4.4		HR 0·41 (0·17–1·00)[c]	OR 0.80 (0.40–1.57)
POPADAD	638/638	Yes	Primary prevention of CV events in patients with type 1 or 2 diabetes and asymptomatic arterial disease	100 mg daily vs. placebo	6.7			OR 0.80 (0.47–1.37)
AAA	1675/1675	Yes	Primary prevention of CVD in individuals at increased CV risk	100 mg daily vs. placebo	8.2			OR 0.86 (0.63–1.17)
PHS	11037/11034	Yes	Primary prevention of CVD and cancer in male physicians	325 mg alternate days vs. placebo	5.0	HR 1.03 (0.83–1.28)	–	–
WHS	19934/19942	Yes	Primary prevention of CVD and cancer in women	100 mg alternate days vs. placebo	9.0	HR 0.80 (0.67–0.97)	–	HR 0.97 (0.88–1.07)

BDAT British Doctors Aspirin Trial, *TPT* Thrombosis Prevention Trial, *SALT* Swedish Aspirin Low-Dose Trial, *UK-TIA* United Kingdom-Transient Ischaemic Attack Aspirin trial, *ETDRS* Early Treatment Diabetic Retinopathy Study, *SAPAT* Swedish Angina Pectoris Aspirin Trial, *JPAD* Japanese Primary Prevention of Atherosclerosis with Aspirin for Diabetes study, *POPADAD* Prevention of Progression of Arterial Disease and Diabetes study, *AAA* Aspirin for Asymptomatic Atherosclerosis trial, *PHS* Physicians' Health Study, *WHS* Women's Health Study, *HR* hazard ratio, *OR* odds ratio

[a]References [25–33]

[b,c]Pooled risk estimates from meta-analyses: [b]reference [24] and [c]reference [34]

surveillance and screening were not available. Thus, bias could have arisen through higher rates of endoscopy and polypectomy for the investigation of bleeding in those taking aspirin. Importantly, because of differences in aspirin-dosing schedule, these meta-analyses did not include the two largest aspirin primary prevention trials to date, the Physicians' Health Study (PHS) [36] and the Women's Health Study (WHS) [37]. The PHS, which randomized 22,071 male physicians to alternate-day aspirin 325 mg or placebo, reported no difference in incident colorectal cancer between groups after the 5-year scheduled treatment period [38], nor after extended follow-up to 12 years [39]. The WHS, which used an alternate-day regimen with an aspirin dose of 100 mg, was the only clinical trial specifically designed to examine the effect of aspirin on the primary prevention of cancer as well as cardiovascular disease [37]. After an average of 10 years of follow-up, randomization to aspirin was not associated with a reduction in the risk of total cancer or colorectal cancer [37]; however, in a subsequent analysis, which included post-trial follow-up through a median of 18 years, a 20 % reduction in incident colorectal cancer was observed in the aspirin group (HR, 0. 80; 95 % CI, 0.67–0.97) [40]. It remains unclear why the PHS and WHS, which both used alternate-day dosing, generated disparate results. The equivalent daily dose of aspirin in WHS is lower than that used in the PHS and some aspirin cardiovascular trials included in the Rothwell meta-analyses. It is of note that the latency period for the development of colorectal cancer has been estimated to be at least 10 years, and it remains possible that the post-trial follow-up in the PHS was still too brief to detect the effect of aspirin on colorectal carcinogenesis in this particular population.

Randomized Controlled Trials of Aspirin for the Prevention of Colorectal Adenomas

Adenomatous polyp occurrence or recurrence is a marker of colorectal cancer risk and is a widely accepted shorter-term intermediate or surrogate outcome measure that can be exploited in chemoprevention studies [41]. In 2009, Cole and colleagues published a meta-analysis of all known trials that had evaluated aspirin's effectiveness in the secondary prevention of colorectal adenomas [42]. The four studies that were included (summarized in Table 14.2) randomized a total of almost 3000 participants, with a recent

Table 14.2 Trials of aspirin in the prevention of colorectal adenomas[a]

Trial	Participants initially randomized	Inclusion criteria	Aspirin dose	Median follow-up (months)	Risk ratio (95 % CI)	
					Any adenoma	Advanced adenoma
APACC	272	Recent history of sporadic colorectal adenomas	160 mg or 200 mg daily vs. placebo	47.2	0.95 (0.75–1.21)	0.91 (0.51–1.60)
ukCAP	939	Recent history of sporadic colorectal adenomas	300 mg daily vs. placebo	37.5	0.79 (0.63–0.99)	0.63 (0.43–0.91)
AFPPS	1121	Recent history of sporadic colorectal adenomas	81 mg daily vs. 325 mg daily vs. placebo	32.2	0.88 (0.77–1.02)	0.74 (0.52–1.06)
CALGB 9270	635	Previous history of resected colorectal cancer	325 mg daily vs. placebo	31.3	0.61 (0.44–0.86)	0.77 (0.29–2.05)
J-CAPP	311	Previous history or sporadic colorectal adenomas	100 mg daily vs. placebo	24.0	0.60 (0.36–0.98)	–

APACC Association pour la Prévention par l'Aspirine du Cancer Colorectal, *ukCAP* United Kingdom Colorectal Adenoma Prevention, *AFPPS* Aspirin/Folate Polyp Prevention Study, *CALGB* Cancer and Leukemia Group B, *J-CAPP* Japan Colorectal Tumor Prevention Study: Randomized Controlled Trial by Low-Dose Aspirin

[a]References [44–48]

history of sporadic adenomas or previous colorectal cancer, to aspirin doses of between 81 mg and 325 mg per day or placebo [42–46]. The analysis was based on 2698 participants who had completed colonoscopic follow-up. The primary endpoint was adenoma occurrence after randomization, while incidence of advanced lesions (adenomas that were $\geq$1 cm in size, contained high-grade epithelial dysplasia or invasive cancer, or featured villous or tubulovillous morphology) served as a secondary endpoint. After median follow-up of 33 months, compared to placebo, the pooled risk estimate for any adenoma at any aspirin dose was 0.83 (95 % CI, 0.72–0.96) and 0.72 (95 % CI, 0.57–0.90) for any advanced lesion [42]. Interestingly, the greatest benefit from aspirin was apparent during the first year after randomization, suggesting that aspirin may exert an effect on early stages of adenomagenesis. In one of the component studies, the Association pour la Prévention par l'Aspirine du Cancer Colorectal (APACC) trial [47], in contrast to the findings at 1 year, no ongoing benefit in adenoma prevention was seen for daily low-dose aspirin after 4 years [43]. It should be noted, however, that the APACC trial was the smallest of the studies included in the meta-analysis and suffered a substantial attrition rate; only 185 of the initial 272 randomized participants underwent colonoscopy at 4 years [43].

All four of adenoma prevention studies included in the meta-analysis by Cole and colleagues were conducted in European or North American populations. Subsequently, however, a multicenter, randomized controlled trial involving 311 Japanese subjects with a history of single or multiple adenomas also reported a reduction in the risk of recurrent adenomas over 2 years in those assigned to aspirin 100 mg daily, compared to placebo (OR, 0.60; 95 % CI, 0.36–0.98) [48]. Interestingly, a greater magnitude of risk reduction was reported for nonsmokers (OR, 0.37; 95 % CI, 0.21–0.68), with an apparent increase in the risk of recurrent adenomas observed among smokers (OR, 3.44; 95 % CI, 1.12–10.64), although the estimates for this stratified analysis are based on relatively small participant and event numbers [48]. In a recent adenoma prevention trial, which found no benefit from aspirin 75 mg daily in combination with calcitriol and calcium, a borderline statistically significant interaction ($P = 0.046$) was observed between smoking and treatment in subgroup analyses according to smoking status [49]. Since smoking has been implicated in "resistance" to the antiplatelet effects of aspirin [50], the possibility of aspirin effect modification by smoking status deserves scrutiny in future studies.

Aspirin Trials in Familial Cancer Syndromes

Further evidence for the antitumor activity of aspirin in humans comes from clinical trials conducted in individuals with the two most common familial colorectal cancer syndromes, FAP and Lynch syndrome (also known as hereditary nonpolyposis colorectal cancer, HNPCC). Classic FAP, which arises due to dominantly inherited mutations in the adenomatous polyposis coli (*APC*) tumor suppressor gene, is characterized by the development of hundreds or thousands of colorectal polyps starting in the second decade of life. Progression to colorectal cancer is inevitable, if not treated by prophylactic colectomy or proctocolectomy, with the average age at colorectal cancer diagnosis being 39 years [51]. Chemoprevention could have a role in delaying the time to prophylactic surgery in some patients with FAP or reducing polyp growth in residual rectal mucosa or in the small intestine. Somatic mutation of *APC* is a common early event in sporadic adenomas [52]. Thus, the results of chemoprevention studies among individuals with FAP may have broader relevance to sporadic colorectal neoplasia.

Early clinical studies in FAP using sulindac [53], and later trials involving the selective COX-2 inhibitors, celecoxib and rofecoxib [54, 55], indicate that these agents are effective in reducing polyp burden. Aspirin was first evaluated in the setting of FAP in the Colorectal Adenoma/Carcinoma Prevention Programme

1 (CaPP1) study, an international, randomized, placebo-controlled trial of aspirin (600 mg/day) and/or resistant starch (30 g/day) over 1–12 years in a two-by-two factorial design [56]. The primary endpoint was polyp number in the rectum and sigmoid colon. Of the 206 patients, aged 10–21 years, who were randomized, 133 had at least one follow-up lower endoscopy. Compared to placebo, individuals in the aspirin intervention arm experienced a statistically nonsignificant reduction in polyp number (RR, 0.77; 95 % CI, 0.54–1.10) and a significant reduction in average maximum polyp size when the analysis was restricted to those who had received treatment for more than 1 year (6.0 mm vs. 3.0 mm, $P = 0.02$) [57]. A Japanese randomized controlled trial has investigated the effect of low-dose aspirin (100 mg/day) compared to placebo in subjects with FAP. Although the target sample size determined by power calculations was 100, only 51 eligible patients were initially identified, of whom only 34 patients went on to complete the trial. Adverse events in three patients receiving aspirin lead the monitoring committee to suspend further recruitment. Since all subjects were already undergoing frequent surveillance and polypectomy, the average size of polyps assessed in the study was around 1.7 mm. After 6–10 months, there was no difference in the primary endpoint of reduction in polyp diameter between treatment and control groups, except in a subgroup analysis of subjects with polyps $\leq$2 mm at the baseline ($P = 0.046$) [58]. The implications of these results are limited by the small sample size and the diminutive size of the polyps, which were evaluated on a submillimetric scale.

The efficacy of aspirin as a chemopreventive agent has also been studied in Lynch syndrome, where autosomal dominantly inherited mutations in genes encoding components of the mismatch repair (MMR) system confer greatly elevated risk of colorectal cancer as well as risk for a spectrum of tumors at extracolonic sites, including the endometrium, stomach, ovaries, small intestine, and urological tract [51, 59]. Microsatellite instability (MSI) is the hallmark of colorectal cancers arising in the context of Lynch syndrome, which accounts for an estimated 3–5 % of all colorectal cancers. The effect of aspirin on tumorigenesis in Lynch syndrome may be relevant to the roughly 15 % of sporadic colorectal cancers that display MSI, commonly as a result of acquired epigenetic silencing of the MMR gene, *MLH1* [60]. The CaPP2 study employed a factorial design similar to that of CaPP1, with a daily aspirin dose of 600 mg. Of 1009 eligible patients, 746 completed the trial and were included in the analysis [61]. A genetically confirmed diagnosis was present in 83 % of participants, with the remainder having a clinical diagnosis of Lynch syndrome. After an average of 29 months of follow-up (27 months of mean treatment duration), there was no difference in colorectal cancer or adenoma incidence between the aspirin and placebo groups [61]. The CaPP2 study included a preplanned double-blind post-intervention follow-up period, and a further analysis was conducted when the earliest recruited participants reached 10 years post-randomization [62]. At this point, the average follow-up was 55.7 months, and 48 participants had developed a first colorectal cancer despite standard surveillance (18 of 427 assigned aspirin and 27 of 329 assigned aspirin placebo). In an intention-to-treat analysis, a nonsignificant trend toward reduced cancer incidence in the aspirin group was observed (HR, 0.63; 95 % CI, 0.35–1.13) [62]. Since the original CaPP2 protocol had specified an intervention of two years of duration [56], an analysis was performed including only those who had consumed a minimum of 1400 aspirin tablets (rounded down from the equivalent of two 300 mg tablets per day for two years). In this per-protocol analysis, aspirin treatment significantly reduced colorectal cancer incidence (HR, 0.41; 95 % CI, 0.19–0.86) and, in a planned secondary analysis, also reduced the risk of any Lynch-associated cancer (HR, 0.45; 95 % CI, 0.26–0.79) [62].

The CaPP3 study, for which recruitment has already started in the UK, is a dose inferiority trial that plans to randomize 3000 participants with Lynch syndrome to 100 mg, 300 mg, or 600 mg of aspirin per day for two years, followed by 100 mg daily for all. The study will collect

participant blood samples to evaluate potential biomarkers of aspirin response and is expected to run until at least 2021 [63].

Aspirin Use Following Colorectal Cancer Diagnosis

One adenoma prevention study, the Cancer and Leukemia Group B (CALGB) 9270 trial, demonstrated that, compared to placebo, 325 mg of aspirin daily over median follow-up of 30.9 months lead to a 35 % reduction in incident adenomas in patients with a history of colorectal cancer resection [45]. Although these data suggest that aspirin prevents recurrent colorectal neoplasia after colon cancer diagnosis, and data from cardiovascular trials have demonstrated a reduced risk of colorectal cancer metastasis among those assigned to aspirin, there are currently no randomized trial data on adjuvant aspirin and recurrence or survival following colorectal cancer diagnosis. Several observational studies have, however, explored this question. In an analysis of 1279 men and women with nonmetastatic colorectal cancer enrolled in the NHS and HPFS cohorts, regular aspirin use after diagnosis, compared to nonuse, was associated with a 29 % reduction in the risk of colorectal cancer-related death over median follow-up of 11.8 years (HR, 0.71; 95 % CI, 0.53–0.95) [64]. In a subgroup analysis of NHS and HPFS participants with tumor tissue available for immunohistochemical assessment, aspirin use was associated specifically with reduced mortality in individuals whose primary tumors overexpressed COX-2 (HR 0.39 and 95 % CI, 0.20–0.76, compared to HR 1.22 and 95 % CI, 0.36–4.18, for COX-2 negative tumors; $P_{\text{interaction}} = 0.04$) [64]. A later analysis, also conducted using data from the NHS and HPFS cohorts, suggested that the reduction in mortality associated with aspirin was restricted to the 10–20 % of individuals whose colorectal cancers harbored a mutation in the gene encoding phosphatidylinositol-4,5-bisphosphonate 3-kinase, catalytic subunit alpha (*PIK3CA*), which leads to upregulation of PI3K activity [65]. Among 964 all-stage colorectal cancer cases, compared to no aspirin use, post-diagnostic regular use of standard-dose aspirin was associated with a mortality HR of 0.18 (95 % CI, 0.06–0.61) for *PIK3CA*-mutated cancers, but was not associated with colorectal cancer mortality among those with *PIK3CA* wild-type cancers (HR, 0.96; 95 % CI, 0.69–1.32) [65]. Similar results were obtained from a post hoc analysis of data from 896 participants in the Vioxx in Colorectal Cancer Therapy: Definition of Optimal Regime (VICTOR) trial of adjuvant rofecoxib compared to placebo [66]. Among individuals with *PIK3CA*-mutated tumors, compared to no aspirin use, the use of low-dose aspirin was associated with a reduction in the risk of colorectal cancer recurrence 0.11 (95 % CI, 0.001–0.832) [66]. In both studies, the number of aspirin users with *PIK3CA*-mutated tumors was limited (66 and 14, respectively), and event numbers were small. In a survival study of 999 colorectal cancer patients from the Eindhoven Cancer Registry, no interaction was observed between post-diagnostic aspirin use and *PIK3CA* mutation [67]. Similarly, a lack of association between *PIK3CA* mutation and survival according to aspirin use was reported in an analysis of 1487 colorectal cancer patients from two Australian hospital-based cohorts [68]. A major limitation of this analysis is the fact that aspirin use was defined as exposure at the time of diagnosis, rather than after diagnosis.

In a recent meta-analysis of aspirin use and colorectal cancer survival, which included seven cohort studies of pre-diagnostic aspirin use and a similar number of cohort studies of post-diagnostic aspirin use, an overall survival benefit was observed for post-diagnostic aspirin use (HR, 0.84; 95 % CI, 0.75–0.94), but not for aspirin use before diagnosis (HR, 1.01; 95 % CI, 0.96–1.06) [69]. No association was observed between pre- or post-diagnostic aspirin use and colorectal cancer-specific mortality; however, only three of the seven post-diagnostic aspirin use studies included this as an endpoint [69].

A meta-analysis of studies that included stratification by tumor *PIK3CA* status reported that the association between aspirin use and colorectal cancer survival was restricted to individuals with *PIK3CA*-mutated tumors, although the authors of the analysis conceded that the number of available studies remains too small to make any definitive conclusions [70].

A number of imminent or ongoing clinical trials will investigate the benefit of adjuvant aspirin among colorectal cancer patients. The ADD-Aspirin double-blind, randomized controlled trial aims to recruit around 11,000 patients from the UK and India who have had potentially curative treatment for breast, colorectal, esophageal, gastric, or prostate cancer [71]. After an 8-week active run-in period on aspirin 100 mg daily, participants will be randomized to continue on aspirin at a dose of 100 mg or 300 mg daily or receive placebo for 5 years [71]. The primary outcome measure for colorectal cancer will be disease-free survival, and follow-up beyond 5 years will be possible using registry data. The Aspirin for Dukes C and High Risk Dukes B Colorectal Cancers (ASCOLT) trial is an ongoing study based in Singapore that anticipates eventual enrollment of 1200 predominantly Asian colorectal cancer patients [72]. Participants are randomized to aspirin 200 mg daily or placebo for 3 years, with disease-free survival as the primary endpoint and overall mortality at 5 years serving as a secondary endpoint. The estimated trial completion date is late 2021. Given the conflicting results from observational studies, prospective evaluation of the predictive capacity of *PIK3CA* mutation is essential. A Swiss multicenter clinical trial, which is due to commence recruitment in October 2015, plans to randomize 185 eligible patients with *PIK3CA*-mutated stage II or III colorectal cancers to aspirin, 100 mg daily, or placebo for 3 years. The completion date for the primary endpoint of disease-free survival is late 2018. Subject to funding, the ADD-Aspirin study also plans to assess tumor *PIK3CA* mutation status during the run-in period and use this as a stratification factor during randomization [71].

Mechanisms of Action of Aspirin in Cancer Chemoprevention

In common with traditional NSAIDs, the central mechanism responsible for the anti-inflammatory effect of aspirin involves inhibition of the prostaglandin-endoperoxide synthase (PTGS) enzyme, more commonly referred to as cyclooxygenase (COX). There are two COX isoforms, and both are selectively acetylated and irreversibly inactivated by aspirin. Most cell types constitutively express the COX-1 (PTGS1) isoform. COX-2 (PTGS2), in contrast, is constitutively expressed only in limited number of tissues, but can be rapidly induced by a variety of stimuli including tissue injury, hypoxia, growth factors, cytokines, and activated oncogenes [73]. The COX enzymes catalyze the conversion of arachidonic acid to prostaglandin (PG) H_2, which is the rate-limiting step in the generation of prostanoids such as PGE_2, PGI_2, and thromboxane (TX) A_2 [74]. The conversion of PGH_2 to prostaglandins and other biologically active mediators is achieved by tissue-specific isomerases.

COX-2 is overexpressed in around 80 % of colorectal cancers and is upregulated at an early stage in a proportion of adenomas [75]. Most hypotheses relating to the chemopreventive mechanisms of aspirin have therefore tended to focus on COX-related pathways and COX-2 in particular [76]. Among the COX-2 metabolites present in colorectal cancer tissues, the pro-inflammatory prostanoid, PGE_2, is the most abundant and appears to act in an autocrine and paracrine fashion to modulate neoplastic cellular attributes such as proliferation, resistance to apoptosis, migration, and invasion [77]. PGE_2 has also been identified as a driver of tumor-associated angiogenesis and is implicated in colorectal cancer metastasis [78–80]. Accumulating evidence also points to a critical role for PGE_2 in facilitating tumor evolution by suppressing myeloid cell activation and promoting tumor immune evasion [81].

The ability of PGE_2 to effect a pro-tumorigenic cellular phenotype is likely to

depend on a number of different molecular mechanisms including MEK-ERK and PI3K-AKT signal transduction via epidermal growth factor receptor activation [82, 83], deregulation of Wnt signaling [84, 85], and modulation of gene transcription as a result of aberrant DNA methylation [86]. In $Apc^{min/+}$ and $Apc^{\Delta 716}$ mice, animal models of human FAP, genetic or pharmacologic inactivation of COX-2 markedly reduces the number and size of intestinal polyps [87–89]. Moreover, administration of PGE_2 augments intestinal tumorigenesis in $Apc^{min/+}$ mice [90] and increases colon tumor multiplicity in a rat model of chemical-induced carcinogenesis [91]. Indirect evidence supporting the importance of COX-2 inhibition in colorectal cancer chemoprevention in humans comes from adenoma prevention trials using selective COX-2 inhibitors [41, 92, 93]. Although these drugs effectively prevent adenoma recurrence, coxibs have been reported to have pleiotropic effects involving COX-independent pathways, such as inhibition of AKT pathway signal transduction and altered sphingolipid signaling [94, 95].

Aspirin may also exert an antineoplastic effect on COX-dependent tumorigenesis through mechanisms other than blockade of PG synthesis. Aspirin appears to be capable of transcriptional repression of COX-2 [96] and has been shown to prevent COX-2-peroxidase-mediated activation of co-carcinogens [97]. Furthermore, acetylation of COX-2 by aspirin renders the enzyme capable of generating 15-epi-lipoxin-A4, or "aspirin-triggered lipoxin," which has anti-inflammatory and growth inhibitory properties [98].

COX-2 appears to be a promising target for chemoprevention. However, it remains uncertain whether aspirin's antineoplastic effects in vivo result primarily from inhibition of COX-2. Aspirin has a short plasma half-life of around 20 min, and it has been estimated that aspirin is 60–170 times more effective at acetylating COX-1 than COX-2 [99]. Orally administered aspirin is subject to first-pass metabolism in the gut and liver, resulting in negligible systemic bioavailability following low-dose administration [100]. Inhibition of COX-1 in the pre-systemic (i.e., portal) circulation therefore may be an important contributor to the antiplatelet effect of low-dose aspirin [101]. Although inhibition of COX in anucleate platelets is irrecoverable, nucleated cells can overcome COX inhibition within 2–4 h by regenerating COX enzymes. Thus, although once-daily low-dose aspirin appears sufficient to influence colorectal cancer risk [35], it seems doubtful that this is achieved by sustained inhibition of systemic COX-2 alone.

Several COX-independent mechanisms have been proposed to account for the antitumor effect of aspirin; these include activation of NFkB [102], direct interference with Wnt or MEK signaling [103–105], interaction with cell cycle regulators [106, 107], disruption of mitochondrial and proteasome function [108, 109], acetylation of non-COX proteins [110], enhanced catabolism of polyamines [111, 112], and attenuation of MMR deficiency [113]. Data supporting these alternative mechanisms derive almost entirely from in vitro experiments, which generally require high concentrations of aspirin, several orders of magnitude greater than peak plasma levels achieved after ingestion of standard therapeutic doses.

Could inhibition of COX-1 therefore be relevant to aspirin's chemopreventive effect? It is interesting that genetic inactivation of COX-1 is as effective as COX-2 knockout in reducing tumor burden in $Apc^{min/+}$ mice [88]. In humans, daily administration of low-dose (81 mg) aspirin, which is considered inadequate to inhibit peripheral COX-2, can reduce PGE_2 levels in colonic mucosal biopsies taken over 3 days after the last dose of aspirin [114]. While this might suggest the involvement of COX-1 inhibition, one would expect once-daily aspirin dosing to only transiently inactivate COX-1 in nucleated colonic epithelial cells. Furthermore, over the course of several days, much of the colonic epithelium itself will have been regenerated. The only cell type susceptible to durable inhibition of COX-1 over this time frame is platelets, and it has thus been hypothesized that the antineoplastic and cardiovascular effects of aspirin might share a common mechanism [115].

The role of platelets in cancer metastasis has been appreciated for several decades [116, 117];

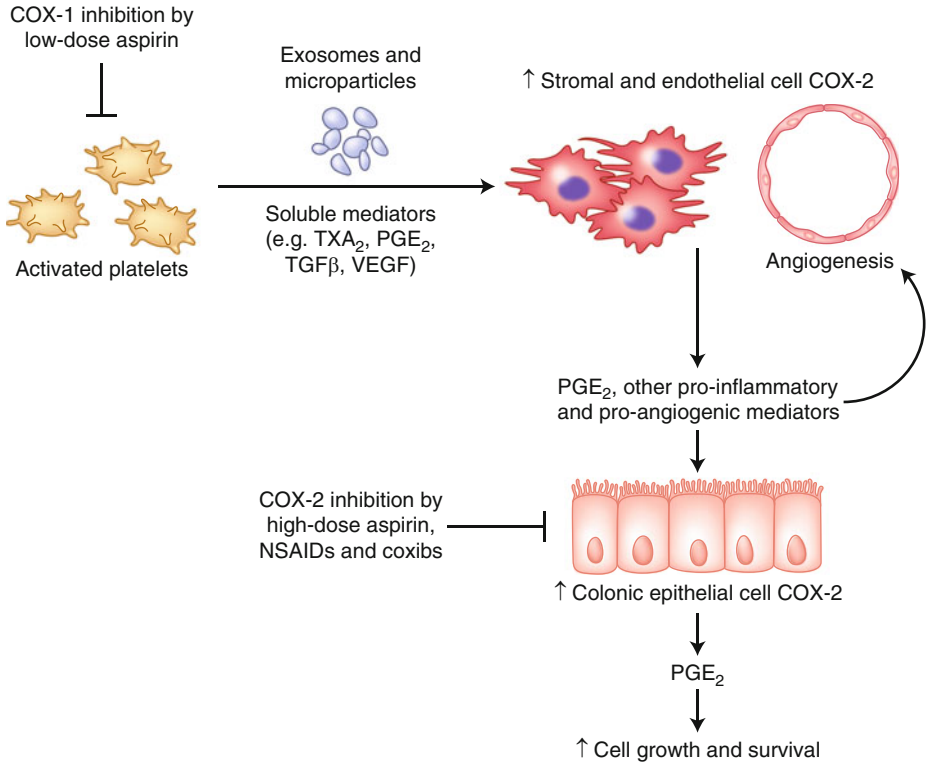

Fig. 14.1 Proposed mechanism through which inhibition of platelet COX-1 by low-dose aspirin can influence colorectal tumorigenesis [119, 120]. Platelet activation generates soluble mediators, microparticles, and exosomes, which alter the behavior of adjacent nucleated cells. Induction of COX-2 expression in stromal and endothelial cells in the colorectal mucosal tissue microenvironment leads to the release of prostanoids and other pro-inflammatory mediators that, in turn, induce COX-2 expression in epithelial cells. Epithelial COX-2 expression generates PGE_2, which, along with mediators from stromal and endothelial cells, promotes cell growth, resistance to apoptosis, and angiogenesis. Abbreviations: *COX* cyclooxygenase, *NSAID* nonsteroidal anti-inflammatory drug, *PGE_2* prostaglandin E_2, *TGF-β* transforming growth factor beta, *TXA_2* thromboxane A_2, *VEGF* vascular endothelial growth factor

however, it has been suggested more recently that platelets could influence earlier phases of tumorigenesis by contributing to chronic inflammation [118]. A model has been proposed whereby activated platelets act as a source of inflammatory mediators that induce COX-2 expression in non-epithelial cells in the mucosal tissue microenvironment (Fig. 14.1) [119, 120]. Platelet-induced COX-2 expression by stromal and endothelial cells then acts as a source of PGE_2 that promotes epithelial cell transformation and growth [119, 120]. This model is attractive since it accommodates both platelet COX-1 and tumoral COX-2 and PGE_2-dependent mechanisms. Activated platelets have the potential to influence the behavior of other cells through direct contact, via the release of soluble pro-inflammatory, growth promoting, and pro-angiogenic molecules, including PGE_2, transforming growth factor beta (TGF-β), and vascular endothelial growth factor [121], or by shedding microparticles and exosomes [121, 122]. Platelets have been shown to be capable of inducing COX-2 expression in the colorectal cancer cell line, HT29, during co-culture [123], and evidence from a mouse model of metastasis suggests that platelet-derived TGF-β can induce epithelial-mesenchymal transition and promote tumor cell metastasis [124]. These data derive from experimental conditions where there is direct contact between platelets and neoplastic cells, such as

might occur during vascular transit. Data supporting an influence of platelets on earlier stages of tumor evolution are currently limited [118].

Clinical Considerations in Adopting Aspirin for CRC Chemoprevention

Aspirin Toxicity

Toxicity associated with regular aspirin use represents the major factor that has limited the recommendation of aspirin for cancer chemoprevention [125]. Among the adverse events associated with regular aspirin use, serious bleeding-related complications, comprising gastrointestinal and intracranial hemorrhage, are the most clinically important. For regular use of aspirin at standard doses (> 325 mg/day), the risk of upper GI bleeding appears to increase in a dose-dependent manner [126, 127]. Evidence for a dose-response relationship for low-dose aspirin (75–325 mg/day) is conflicting [128–133]. Nonetheless, low-dose aspirin has consistently been found to increase GI bleeding risk. In a meta-analysis of 35 randomized controlled trials, compared to control agents, daily low-dose aspirin increased the risk of major GI bleeding by 55 % (HR, 1.55; 95 % CI, 1.27–1.90), equivalent to an additional one to two significant GI bleeds per 1000 person-years [134]. Data from epidemiologic studies and randomized trials suggest that the elevated GI bleeding risk associated with aspirin use diminishes with increasing time since the initiation of therapy [135, 136]. In a meta-analysis of six primary prevention trials of daily low-dose aspirin, no excess major extracranial bleeding was observed in the treatment group when the follow-up period was restricted to ≥3 years [136]. Furthermore, the case fatality rate for major extracranial bleeding across these trials was lower for individuals on aspirin compared to controls, suggesting a protective effect of aspirin on death from major extracranial bleeding (OR, 0.32; 95 % CI, 0.12–0.93) [136]. The GI bleeding risk associated with long-term low-dose aspirin may be partly mitigated by *H. pylori* eradication [137, 138], which could potentially reduce upper GI complications of aspirin therapy in the general population by up to 30 % [139]. The UK-based Helicobacter Eradication Aspirin Trial (HEAT) aims to recruit in excess of 6000 *H. pylori*-positive individuals (≥60 years of age) who are taking low-dose aspirin [140]. The study will address whether eradication therapy, compared to placebo, is effective in preventing ulcer bleeding complications. Concomitant administration of proton pump inhibitors (PPIs) has been estimated to reduce upper GI complications of aspirin therapy by 66 % in a meta-analysis of three randomized trials [134]; however, the overall benefit and cost effectiveness of this approach in the general population remain uncertain [141].

Although intracerebral and subarachnoid hemorrhage attributable to aspirin use is relatively rare, it is considered the most serious complication of aspirin therapy on account of the associated risk of death or long-term disability [142]. A meta-analysis by the Antithrombotic Trialists' (ATT) Collaboration, which included individual participant data from six primary prevention trials and 16 secondary prevention trials of low-dose aspirin, found that aspirin increased the relative risk of intracranial bleeding by 39 % (RR, 1.39; 95 % CI, 1.08–1.78), which translates to an absolute risk of one or two excess bleeds per 10,000 patient-years [143]. Hypertension is a major risk factor for intracranial bleeding, and it has been proposed that adequate blood pressure control may reduce the risk associated with aspirin use [142]. In the Hypertension Optimal Treatment (HOT) study, which enrolled hypertensive subjects to targeted blood pressure reduction, there was no difference in the rate of intracranial bleeding between the aspirin and control groups after achieving blood pressure control [144].

Based on data from randomized trials, the absolute risk of major bleeding associated with low-dose aspirin use appears modest; however, data from a population-based cohort study suggest that the "real-world" hemorrhagic risks associated with aspirin may have been underestimated [145]. In the analysis, which

utilized administrative data from 12 regional health authorities in Puglia, Italy, over 186,000 individuals being prescribed low-dose aspirin were propensity sore-matched 1:1 to controls who did not take prescribed aspirin [145]. Among aspirin users, the incidence of major bleeding was around fivefold higher than estimates obtained from meta-analyses of randomized trials (incidence rate ratio, 1.55; 95 % CI, 1.48–1.63) [145]. The rate of bleeding in controls in this population was also considerably higher than that observed in clinical trials, which may reflect differences in the prevalence of other bleeding risk factors such as hypertension and the use of non-aspirin NSAIDs [145]. It has therefore been suggested that results from the ATT Collaboration's meta-analysis [143] remain the most robust risk estimates for general populations in Europe and North America [146].

Dose and Duration of Treatment

Since the adverse effects of aspirin appear to be largely dose related, at least for standard doses (>325 mg/day), it is important to establish the smallest dose of aspirin capable of effectively preventing colorectal neoplasia. In the meta-analysis of long-term trial follow-up data by Rothwell and colleagues [24], there appeared to be no difference in the effectiveness of lower doses of aspirin (75 mg–300 mg/day) compared to higher doses (500–1200 mg/day). Data on cancer outcomes from trials including head-to-head comparisons of aspirin doses are limited [28, 147]; however, follow-up of participants in the Dutch TIA study [147] showed an increased risk of fatal colorectal cancer in the group who received a very low-dose of aspirin, 30 mg/day, compared to those who were assigned to 283 mg/day [24]. These findings suggest that long-term daily aspirin doses of at least 75 mg are required to prevent colorectal cancer incidence and mortality. For alternate-day dosing, analysis of extended follow-up in the WHS demonstrated a reduction in the risk of incident CRC with 100 mg of aspirin [40], although men assigned 325 mg on alternate days did not experience a reduction in CRC risk over 12 years of follow-up in the PHS [39].

Observational data tend to suggest that higher aspirin doses, ≥300 mg/day, might be necessary to prevent incident colorectal cancer [148], although many of these studies captured limited information on aspirin dose and duration of use. In the NHS and HPFS prospective cohorts, where data on aspirin use frequency is available over a prolonged period, the greatest reduction in CRC risk was associated with the maximum use category of 14 or more standard aspirin tablets per week [19, 20].

In meta-analysis of adenoma prevention trials, which employed aspirin doses between 81 mg and 325 mg/day [42], lower doses (≤160 mg/day) resulted in a reduction in the risk of recurrent adenoma that was of comparable magnitude to that observed for any aspirin dose [42]. Comparison of higher aspirin doses (≥300 mg/day) to placebo also demonstrated a statistically significant reduction in absolute risk for any adenoma. However, in a pooled analysis of the two studies that directly compared lower-dose to higher-dose aspirin, significantly greater risk reduction was found with low-dose aspirin [42]. This atypical dose-response relationship precludes firm conclusions about the relative effectiveness of lower vs. higher doses of aspirin for adenoma prevention.

The results of two ongoing multinational, placebo-controlled, primary prevention trials of low-dose aspirin (100 mg/day), the Aspirin in Reducing Events in the Elderly (ASPREE) [149] and the Aspirin to Reduce Risk of Initial Vascular Events (ARRIVE) [150], may provide additional evidence for low-dose aspirin in colorectal cancer chemoprevention, although the scheduled follow-up duration in both trials is only five years. In addition, the outcome of the CaPP3 trial may be extrapolated to help inform choice of aspirin dose for sporadic colorectal cancer prevention [63].

Randomized trials and observational studies have consistently shown that there is a duration-risk relationship between aspirin use and CRC [148]. In analyses of data from the UK-TIA and BDAT studies, reduction in CRC incidence was

observed after a latency period of around 10 years following assignment to aspirin for 5 or more years ($P_{interaction} = 0.004$ for aspirin and follow-up time in the UK-TIA) [148]. In the UK-TIA study [28], no unblinding was performed at the end of the 5-year treatment period, and, since participants would have been unaware of their study assignment, one would not expect there would be significant differences in self-selected use of aspirin post-trial. Thus, the reduction in CRC incidence and mortality observed beyond 10 years post-randomization is likely be attributable to aspirin taken for 5 years during the study period [148]. These findings are generally consistent with data from additional randomized trials and observational studies [24, 148].

Tumor Location

A number of observational studies have suggested that aspirin, or non-aspirin NSAIDs, may have differential associations with CRC risk depending on tumor location, with some studies reporting stronger associations for proximal colon cancer risk and weaker or nonexistent associations for distal colon and rectal cancer risk [22, 151, 152]. Site-specific associations are inconsistent across the literature, and in a meta-analysis of 19 case-control studies and 11 cohort studies, no convincing differential associations were observed for aspirin and CRC risk according to tumor location, age, sex, race, or family history [148]. In the meta-analysis of long-term individual data from four randomized trials by Rothwell and colleagues, assignment to daily aspirin for an average of 5.8 years reduced the 20-year risk of colon cancer (HR, 0.76; 95 % CI, 0.60–0.96), but not rectal cancer (HR, 0.90; 95 % CI, 0.63–1.30) [24]. Where data on colonic subsite were available, aspirin reduced the risk of proximal (HR, 0.45; 95 % CI, 0.28–0.74), but not distal (HR, 1.10; 95 % CI, 0.73–1.64; $P_{difference} = 0.04$) colon cancer [24]. When analyses were restricted to participants with a treatment duration of at least 5 years, a 70 % reduction in the risk of proximal colon cancer was observed in addition to a statistically significant reduction in rectal cancer risk (HR, 0.58; 95 % CI, 0.36–0.92); however, no effect on the incidence of distal colon cancer was observed [24]. Thus, the magnitude of benefit from aspirin may differ according to tumor anatomic location as well as duration of use.

Overall Risk-Benefit of Aspirin and Strategies to Personalize Chemoprevention

Any decision to recommend regular aspirin for primary disease prevention must take into account the balance of absolute risks and benefits, including effects on total cancer incidence and mortality. In a recent analysis of prophylactic aspirin use, modeled using data from the UK population, 10 years of aspirin use starting at age 50, 55, 60, or 65 years was associated with a consistently favorable benefit-harm profile over a 15- to 20-year period [146]. The net absolute mortality reduction associated with commencing aspirin at age 55 years was 1.43 % for men and 0.7 % for women, with almost all of this benefit (89 %–96 %) resulting from prevention of deaths from cancer [146]. It has been suggested that the estimates for aspirin-associated harms used in this analysis were excessively high and failed to take into account the diminution in the risk of extracranial bleeding that occurs over time [153]. Since the study authors aimed to use conservative estimates of harm, it is possible that the actual net benefits of aspirin use in the general population may be greater than their predictions. Even in the absence of increased cardiovascular risk, the beneficial effect of aspirin on cancer mortality makes it possible that aspirin prophylaxis would be cost saving or cost-effective, at least in men [154].

In 2007, the US Preventive Services Task Force recommended against the routine use of aspirin or NSAIDs for colorectal cancer prevention, citing a lack of evidence and concerns over toxicity [125]. Having recently reevaluated the available evidence, the USPSTF has put forward

a draft proposal that recommends 10 years of low-dose aspirin use for combined cardiovascular and cancer prevention among individuals aged 50–59 years who have a 10-year cardiovascular risk of >10 % and are not at increased risk for bleeding complications [155]. This is a significant step forward from the previous USPSTF position statement and will no doubt lead to an increase in aspirin use in the general population. There will, however, be a proportion of the population who stand to benefit from the cancer preventive effects of aspirin, but who do not fulfill the cardiovascular risk criteria. It would therefore be desirable to be able to personalize chemoprevention by stratifying individuals according to their predicted benefit from aspirin.

Several metabolic and genetic markers have recently emerged that may facilitate personalized CRC chemoprevention with aspirin. 15-Hydroxyprostaglandin dehydrogenase (15-PGDH) acts as a metabolic "brake" on PGE_2 synthesis [156]. 15-PGDH null mice are resistant to the antineoplastic effects of celecoxib, and, among 16 participants in the Adenoma Prevention with Celecoxib (APC) trial, higher pretreatment mucosal 15-PGDH predicted response to celecoxib [156]. In an analysis of a subset of participants from the NHS and HPFS cohorts, higher normal mucosal 15-PGDH expression was associated with a >50 % reduction in CRC risk with regular aspirin use, but there was no association between aspirin and CRC risk for those with lower levels of 15-PGHD expression [157]. Prostaglandin E metabolite (PGE-M), the major urinary metabolite of PGE_2, has also been proposed as a metabolic biomarker of colorectal neoplasia. In a nested case-control study within the NHS, aspirin use was associated with a reduced risk of colorectal adenoma only among participants with urinary PGE-M concentrations in the highest three quartiles [158]. In contrast, no association between urinary PGE-M and adenoma recurrence according to aspirin assignment was observed in the Aspirin/Folate Polyp Prevention Study (AFPPS) [159]. Thus, additional large prospective analyses are required to further evaluate PGE-M as a potential predictive marker of aspirin responsiveness.

In a recent genetic association study, exploiting the databases of the Colon Cancer Family Registry and Genetics and Epidemiology of Colorectal Cancer Consortium, two common genetic polymorphisms demonstrated interaction with aspirin use status in their associations with CRC risk [160]. Compared to nonusers, a reduced risk of CRC was associated with aspirin or NSAID use among those with the most common, AA, genotype of rs2965667, at 12p12.3, whereas increased CRC risk was observed with aspirin use in the minority of individuals with AT or TT genotypes ($P_{\text{interaction}} = 4.6 \times 10^{-9}$) [160]. Similarly, the commonest, AA, genotype of rs16973225, at 15q25.2, was associated with reduced CRC risk among aspirin or NSAID users; however, the minor, AC and CC, genotypes were not associated with differential CRC risk according to aspirin or NSAID use [160]. While the functional significance of these variants remains unknown, a possible mechanism for rs2965667 might relate to its proximity to the microsomal glutathione S-transferase 1 gene (*MGST1*), a xenobiotic metabolizing enzyme that has high sequence homology with PGE_2 synthase and whose activity confers cellular resistance to oxidative stress [161]. It is also notable that rs16973225 polymorphism is located downstream of the gene encoding the pro-inflammatory cytokine, interleukin 6, which has been implicated in the pathogenesis of colorectal cancer [162]. It is possible that these two genetic variants could help identify a minority of individuals for whom aspirin use is ineffective or harmful; however, validation in additional populations is required.

Conclusions

The balance of evidence is shifting in favor of aspirin as an agent for the chemoprevention of colorectal neoplasia. This trend toward the acceptance of aspirin for broader indications, beyond cardiovascular prophylaxis, is reflected in the recent USPSTF draft recommendation. Nonetheless, many areas of uncertainty remain that are fundamental to the adoption of aspirin

for population-based CRC chemoprevention. Most important among these are the optimal dose and duration of aspirin therapy, which have yet to be defined. It is not clear what the durability or persistence of aspirin's protective effect is and at what age aspirin should be discontinued for maximal net benefit. Although ongoing clinical studies of aspirin, combined with accumulating data from post-trial follow-up of completed randomized trials, may help shed light on some of these areas of uncertainty, the prospect of a large randomized trial of aspirin for CRC primary prevention seems highly unlikely given the long follow-up that would be required, the already high prevalence of aspirin use with the potential for "drop-in" off protocol use of aspirin, and the attendant logistical and cost issues. Elucidating molecular mechanisms that participate in the chemopreventive effect of aspirin at physiologically relevant doses remains a crucial research goal. Defining these mechanisms may help inform the optimal dosing for cancer prevention and could yield targets for synergistic chemopreventive approaches in combination with other agents.

A further area of uncertainty is whether there are subgroups of the population who do not stand to benefit from aspirin use. A recent analysis conducted using data from the WHS suggests that alternate-day low-dose aspirin may be ineffective or harmful for the majority of women aged $\geq$45 years of age [163]. Furthermore, the impact of aspirin use on CRC incidence and mortality in individuals who are already participating in colorectal screening is not known. The identification of predictive biomarkers of benefit and harm from aspirin therapy should also, therefore, be a research priority as we strive to develop precision colorectal cancer chemoprevention strategies.

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